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The acute and incremental thermal tolerance of Atlantic cod (*Gadus morhua*) families under normoxia and mild hypoxia☆

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ABSTRACT

Given climate change projections, the limited ability of fish reared in sea-cages to behaviourally thermoregulate, and that thermal tolerance may be heritable, studies that examine family-related differences in upper thermal tolerance are quite relevant to the aquaculture industry. Thus, we investigated the upper thermal tolerance of 15 Atlantic cod (*Gadus morhua* L.) families by challenging them with acute (2 °C h^{-1}) and incremental (1 °C every 4 days) temperature increases (CT_{max} and IT_{max} tests, respectively) under normoxia (~ 100% air saturation) and mild hypoxia (~ 75% air sat.). The cod's CT_{max} was 22.5 ± 0.1 °C (mean ± S.E.) during normoxia and 21.8 ± 0.1 °C during hypoxia ($P < 0.001$); and these two CT_{max} values were significantly correlated across families. In both the normoxic and hypoxic IT_{max} tests, feed intake fell by ~50% between 17 and 18 °C, and stopped entirely by 21 °C. No mortalities were observed under 20 °C in the normoxic and hypoxic IT_{max} tests, and the IT_{max} value was ~21.7 °C in both groups. Differences in the upper thermal tolerance between families were only observed in the CT_{max} experiment. No correlation was found between the specific growth rate and the CT_{max} of the families. Further, no correlation existed between CT_{max} and IT_{max}. This study is the first to compare the thermal tolerance of fish families to both CT_{max} and IT_{max} challenges, and the data: 1) suggest that the Atlantic cod is quite tolerant of acute (i.e., hours) or short-term (i.e., weeks) exposure to high water temperatures (i.e., up to 20 °C); 2) indicate that it might be difficult to select fish with higher IT_{max} values; and 3) question the relevance of CT_{max} for selecting fish that are destined for sea-cages where temperatures slowly warm over the summer.

1. Introduction

Marine temperatures are predicted to increase by as much as 2–4 °C over the next 100 years (IPCC, 2013), and impact the growth performance, physiology and distribution of many aquatic species (Ficke et al., 2007; Portner and Peck, 2010; Butzin and Portner, 2016; Shultz et al., 2016). However, the effect of climate change on aquaculture production and capture fisheries is uncertain as while tropical regions are expected to experience lower productivity, high latitude countries may benefit from increases in production (Doney et al., 2012; Merino et al., 2012; Bell et al., 2013). For example, Butzin and Portner (2016) combined physiological modelling with climate change projections, and showed that Atlantic cod (*Gadus morhua* L.) may exhibit an increase or decrease in maximum weight depending on their geographical location. Although it is not possible to accurately predict how climate change will affect fish production, the impact of changes at multiple levels of biological organization may pose a substantial challenge for aquaculture and may negatively impact the sector (Brander, 2007; Frost et al., 2012), as well as, bring some opportunities to the industry (Sae-Lim et al., 2017).

Recent evidence indicates that marine ectothermic organisms, including fish, are shifting towards higher latitudes as a result of increased seawater temperatures (Fogarty et al., 2017; Kleisner et al., 2017; Alabia et al., 2018). While wild fishes are able to move to higher latitudes, fish held in sea-cages are unable to escape diurnal or seasonal changes in water temperature. For example, cage-cultured cod face large seasonal (0 to 20 °C) and daily (± 10 °C, e.g. with inversion of the thermocline) fluctuations in temperature (Gollock et al., 2006; Björnsson et al., 2007), and mortalities have been associated with warm summer water temperatures. Reductions in dissolved oxygen levels are

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also predicted to accompany periods of high water temperature due to a number of factors. These include increased oxygen demand by the fish, algal blooms, increased net fouling, heightened microbial respiration, reduced water oxygen solubility, and a reduced rate of oxygen resupply from the atmosphere to the ocean interior (Breitburg et al., 2018). It is not uncommon, for example, for dissolved oxygen levels to reach 75% of air saturation and they may get as low as 30% (air sat.) at aquaculture sites if all of the above factors coincide (e.g., Burt et al., 2012; Stehfest et al., 2017). In addition, according to the oxygen and capacity limited thermal tolerance (OCLTT) concept, fish will likely have a lower upper thermal tolerance under conditions of hypoxia (Pörtner, 2002, 2010; Pörtner et al., 2017).

Despite the information above, the selection of families (selective breeding) to cope with the impacts of climate change on aquaculture production has not been extensively explored, as recently discussed by Sae-Lim et al. (2017). Further, few studies to date have investigated intra-specific differences in fish upper thermal tolerance (Meffe et al., 1995; Doyle et al., 2011; Eliason et al., 2011; Anttila et al., 2013). However, Dahlke et al. (2016) recently showed that paternity affects the thermal tolerance of early life-stage Atlantic cod, and suggested that genetic variation underlying thermal reaction norms may allow for the favorable selection of cod populations exposed to environmental changes such as ocean warming.

Given climate change projections for increases in water temperatures and in the occurrence and severity of hypoxia, and that thermal tolerance in cod appears to have a significant heritable component, we present findings from experiments that assessed the acute (CT_{max}) and incremental (IT_{max}) upper thermal tolerance of a number of Atlantic cod families under conditions of normoxia (~ 100% air saturation) and mild hypoxia (~ 75% air sat.). The latter is an O_2 level that has been observed in cage-sites in Newfoundland during the summer (Burt et al., 2012).

2. Materials & methods

2.1. Experimental animals

These studies were conducted in accordance with the guidelines of the Canadian Council on Animal Care and approved by the Institutional Animal Care Committee of Memorial University of Newfoundland (MUN), Canada (Protocol# 10-14-KG).

This research was a component of the Atlantic Cod Genomics and Broodstock Development Project (CGP). Wild Atlantic cod were caught off Cape Sable, Nova Scotia (Northwest Atlantic Fisheries Organization (NAFO) Division 4X) and transported to the Department of Fisheries and Oceans, St. Andrews Biological Station, Canada. Prior to spawning, broodstock were tagged with Passive Integrated Transponder (PIT, Sokymat, Switzerland) tags. Further, fin clips were taken, the total genomic DNA was isolated from these tissue samples, and the fish were genotyped using a microsatellite marker multiplex [see Garber et al., 2010 for additional details]. This genotyping information was loaded into MER (Wang, 2002) to estimate potential relatedness between each of the broodstock. This information was used at the time of spawning to create families from male and female broodstock that were not related based on the microsatellite marker multiplex.

Production of the 15 families used in this study occurred from 10 February to 03 March 2006. Nine female broodstock and 12 male broodstock were used 1 to 2 times to create half sibling linkages where each family was linked to a minimum of one other family. Fertilized eggs from each family were placed into separate incubators, and at hatch were transferred into separate individual family rearing tanks. At 105 days post-fertilization, each family was standardized to 1500 fish per tank, and the tanks were standardized to 450 fish per tank approximately one month later. Standardizations were completed to reduce potential environmental differences that might arise during individual tank rearing. Fish were PIT tagged from 13 to 20 September (2007) for this study, and for use in other studies and for the breeding nuclei. Overall average weight of the individuals from the 15 families at time of tagging was 26.4 g, and average standard length was 13.6 cm. The families were communally reared after PIT-tagging for short period (~1 week) prior to transfer to the Dr. Joe Brown Aquatic Research Building (JBARB, MUN).

At the JBARB, the fish were kept under normoxic conditions at 10 °C in three 3 m^3 tanks, and fed a ration of 1.5% body weight (BW) each morning for approx. 4 months before they were given an acute thermal (CT_{max}) test (see below). This ration is comparable to the satiation level reported for cod of similar size (Imsland et al., 2005). The specific growth rate (SGR) of the fish during this period was calculated using the formula below; final weight and length were recorded when each fish lost equilibrium at the end its acute thermal challenge (CT_{max} test)

$$SGR = [(\ln(\text{final weight in grams}) - \ln(\text{initial weight in grams})) / t(\text{in days})] \times 100$$

2.2. Acute thermal (CT_{Max}) challenge

The acute thermal challenges were run in 25 trials (13 at normoxia, ~100% air saturation; 12 under conditions of mild hypoxia, ~75% air sat.) using a 330 L tank that was supplied with temperature controlled seawater (~32 ppt salinity) from a reservoir (~500 L volume) at the appropriate level of oxygenation. Temperature in the header was controlled by a custom-made heater/chiller (Memorial University Technical services), and a custom made control system (MUN Tech Services) bubbled air or nitrogen into the reservoir tank as required to maintain water oxygen levels between 95-105% and 70–80% air saturation. The water entered the tank through a vertical spray bar mounted on the side of the tank, and this was used to generate a slow circular current within the tank.

At the start of each trial, ~20 fish (72 ± 18 g; mean ± S.E.; see details in supplementary Table 1) were selected haphazardly from their holding tank, and placed into the tank in which the CT_{max} test would be performed (tank temperature 10 °C). The water O_2 level in this tank was either maintained at normoxic levels, or lowered to ~75% saturation over approximately 4 h (with the fish remaining at this reduced level of oxygenation until the next morning, ~18 h in total). The fish were not fed during this period, and thus, were approximately 24 h post-prandial when the CT_{max} trials began. During the CT_{max} test, the external heater/chiller unit was set so that temperature in the system increased from 10 °C at a rate of 2 °C h^{-1}. The fish were observed throughout this period, and were removed upon losing equilibrium [i.e., reaching their critical thermal maximum, CT_{max}; Becker and Genoway, 1979]. The temperature, weight and length of each fish were recorded at this time, and individual fish were identified to family using their internal PIT tag and had a fin clip taken from the dorsal fin. This sample was used to genotype the fish (see above) to ensure that there had not been genetic contamination during spawning/rearing (i.e., each family only contained fish from a specific male x female mating).

2.3. Incremental thermal (IT_{max}) challenge

Three separate trials were conducted at the JBARB (MUN) after the CT_{max} trials were finished. These experiments were conducted using fish (final mass 141 ± 39 g; mean ± S.E.; see Supplementary Table 2), which were haphazardly selected from the Atlantic cod families that remained in the holding tanks. In each trial, fish were placed into 6 different 330 L tanks (35 fish $tank^{-1}$) supplied with flow-through water, and allowed to recover for 4 days at 10 °C and ~100% air saturation. The flows in the tanks were set at 6.0 L min^{-1}, an air stone within each tank was used to maintain appropriate oxygen levels, and a calibrated digital thermometer was used to accurately monitor the temperature in each tank. After the acclimation period, these tanks

were randomly assigned to one of two treatments: i) normoxia (100 ± 3% air saturation) or ii) mild hypoxia (75 ± 3% air saturation), and hypoxia was induced in three tanks by turning down the air stones and reducing the flow into the tanks from 6.0 L min^{-1} to 2.4–3.0 L min^{-1}. Oxygen saturation was monitored (OxyGuard Handy Polaris, OxyGuard International A/S, Birkerød, Denmark) during this reduction in oxygen level, and air flow was adjusted as needed to maintain the hypoxic conditions. After the appropriate level of hypoxia was achieved, temperature was increased every 4 days by 1 °C until all fish died or became moribund, and were removed from their tank; i.e. reached their incremental temperature maximum (IT_{max}). Temperature and oxygen levels were monitored each morning throughout the trial (which lasted a total of 7–8 weeks), and water ammonia levels were also monitored bi-weekly initially, and once weekly thereafter (these never exceeded 0.02 ppm). Fish in each tank were offered a ration of 1.5% BW day^{-1} each morning, but fish did not consume their entire ration as temperature increased (i.e., it was clear that they had reached satiation prior to receiving 1.5% BW). Food consumption data was recorded daily, and used to examine feed intake. When temperatures reached 19 °C, the tanks were checked frequently (i.e., at least every 4 h), and fish that were dead or moribund were removed from the tank and the date, time, temperature, weight, length, and PIT-tag number were recorded. Due to a facility water shutdown and a resultant inability to control water temperatures during part of IT_{max} trial #2, this data was removed and only trials #1 and #3 were used in the analysis. This resulted in a reduced number of fish for certain cod families, and families # 4 and 22 were removed from the experiment due to low numbers.

Water was heated for all experiments using a large plate heat exchanger, and was degassed prior to entering the header tank that supplied the experimental tanks.

2.4. Data management and statistical analysis

A linear mixed effects model was used to test for the effects of family and oxygen level (fixed factors) on CT_{max}, with the final weight of each fish used as a covariate (i.e., CT_{max} was found to be significantly related to fish weight; see Fig. 1S) and trial number as a random factor. Due to the large number of families used in this experiment, not all pairwise comparisons could be made. Thus, pairwise comparisons were made between the two "best" performing families and the other families with respect to CT_{max}, with P values adjusted according to Scheffé's post-hoc methods. A similar statistical model was used to test for the effects of family and oxygen level on IT_{max}, but in this analysis, both tank and trial number were included as random factors in the model. Pearson's correlation analyses were performed to examine whether there was a relationship between the mean upper thermal tolerance of the families in the two thermal challenges (CT_{max} and IT_{max}) under normoxia or mild hypoxia. Finally, a 2 (CT_{max} and IT_{max}) × 2 (oxygen level) factorial, followed by Scheffé's post-hoc tests, was used to examine the effects of the rate of temperature increase (i.e., CT_{max} vs. IT_{max}) and oxygen level (normoxia vs. hypoxia) on the upper thermal tolerance of the families.

A general linear model (GLM) ANOVA followed by Scheffé's post-hoc tests was used to look for differences in SGR between the Atlantic cod families before they were given the CT_{max} challenge. Again, due to the large number of families used in this experiment, not all pairwise comparisons could be made. Thus, pairwise comparisons were made between the two "best" performing families and the other families with respect to SGR. In addition, a Pearson's correlation analysis was used to examine whether there was a relationship between CT_{max} and SGR.

3. Results

The genotyping revealed that one of the families used in the trials contained individuals from other families not chosen for this study, and thus, this family was removed from the dataset. Thus, the data reported for CT_{max} is only for 14 cod families. In addition, two of the families had no/a limited number of individuals available for the IT_{max} test (as explained previously), and so this test was only run with 12 families.

Atlantic cod challenged with the acute temperature increase alone had an overall CT_{max} of 22.5 ± 0.1 °C, whereas fish also exposed to mild hypoxia for 18 h had a CT_{max} of 21.8 ± 0.1 °C ($P < 0.001$; Figs. 1A and 3). Further, CT_{max} was significantly different between the families ($P < 0.001$; O_2 x family interaction, $P = 0.13$). For example, families 31 and 34 had the highest CT_{max} values (22.6 ± 0.1 °C and 22.8 ± 0.1 °C, respectively; mean ± S.E.) while family 25 only had a CT_{max} of 21.7 ± 0.1 °C (Fig. 1A). Overall, the CT_{max} of families 31 and 34 were significantly higher than that measured in 3 and 7 of the other families, respectively. Interestingly, there was a strong positive relationship between a family's CT_{max} under normoxic and hypoxic conditions (Fig. 2A).

In contrast to the results for CT_{max}, there were no differences in IT_{max} between the families and mild hypoxia had no impact ($P = 0.86$) on this parameter (Figs. 1B and 3). Thus, while the IT_{max} of cod exposed to normoxia was significant lower (by approx. 0.7 °C) as compared to the their CT_{max} ($P < 0.001$; Fig. 3), there was no difference between CT_{max} and IT_{max} under mild hypoxia ($P = 0.873$; Fig. 3) and no correlation existed between IT_{max} and CT_{max} under normoxia or hypoxia (Figs. 2B and D).

SGR differed significantly between the families (GLM ANOVA for families, $F = 16.2$ and $P < 0.001$) before they were given the CT_{max} test, with families 12 and 13 having the highest SGR values (1.07 ± 0.04 and 1.15 ± 0.03% day^{-1}, respectively) (Fig. 4A). However, these families were not those that had the highest acute thermal tolerance (CT_{max}), and no significant correlation was observed between CT_{max} and the SGR of the cod families ($P = 0.92$ for normoxia and $P = 0.43$ for mild hypoxia, Fig. 4B) or when the data for individual fish were plotted (data not shown). Feed intake decreased by approximately 50% from 17 to 18 °C, and stopped altogether by 21 °C. Mild hypoxia had no effect on feed intake during the incremental temperature increase (Fig. 5).

4. Discussion

Relatively few studies have examined how 'family' influences the thermal tolerance of cultured fish species. Here, we evaluated the thermal tolerance of Atlantic cod families by exposing them to acute (CT_{max}) and incremental (IT_{max}) temperature increases. Further, two levels of oxygen saturation (100 and 75% air saturation) were used to examine the impact of mild hypoxia (as seen at sea-cage sites in the summer; Johansson et al., 2007; Burt et al., 2012), if any, on the thermal tolerance of this species. We show that: i) there were differences between the thermal tolerance of cod families exposed to a CT_{max} challenge, and that this difference was as high as 1.4 °C; ii) moderate hypoxia (~ 75% air saturation) had limited (0.7 °C lower CT_{max}) to no (for IT_{max}) influence on the cod's upper thermal tolerance; iii) family did not have a significant effect on IT_{max}; iv) no relationship was found between CT_{max} and IT_{max}; and v) family differences in SGR were not correlated with the cod's CT_{max}.

4.1. CT_{max}, IT_{max}, and the effect of hypoxic exposure

In this study, the mean CT_{max} was 22.5 °C under normoxia and 21.8 °C under mild hypoxia. These data are in agreement with previous work, where Atlantic cod were exposed to an acute thermal challenge and CT_{max} ranged from 22 to 24 °C (Sartoris et al., 2003; Gollock et al., 2006; Perez-Casanova et al., 2008a; Gamperl et al., 2009). IT_{max} was found to be 21.8 °C under normoxia and 21.7 °C under mild hypoxia. These data are somewhat higher than reported by Perez-Casanova et al. (2008b) who found that the cumulative mortality of normoxic Atlantic cod juveniles was 73.3% by 19.1 °C during an IT_{max} trial. The fish were

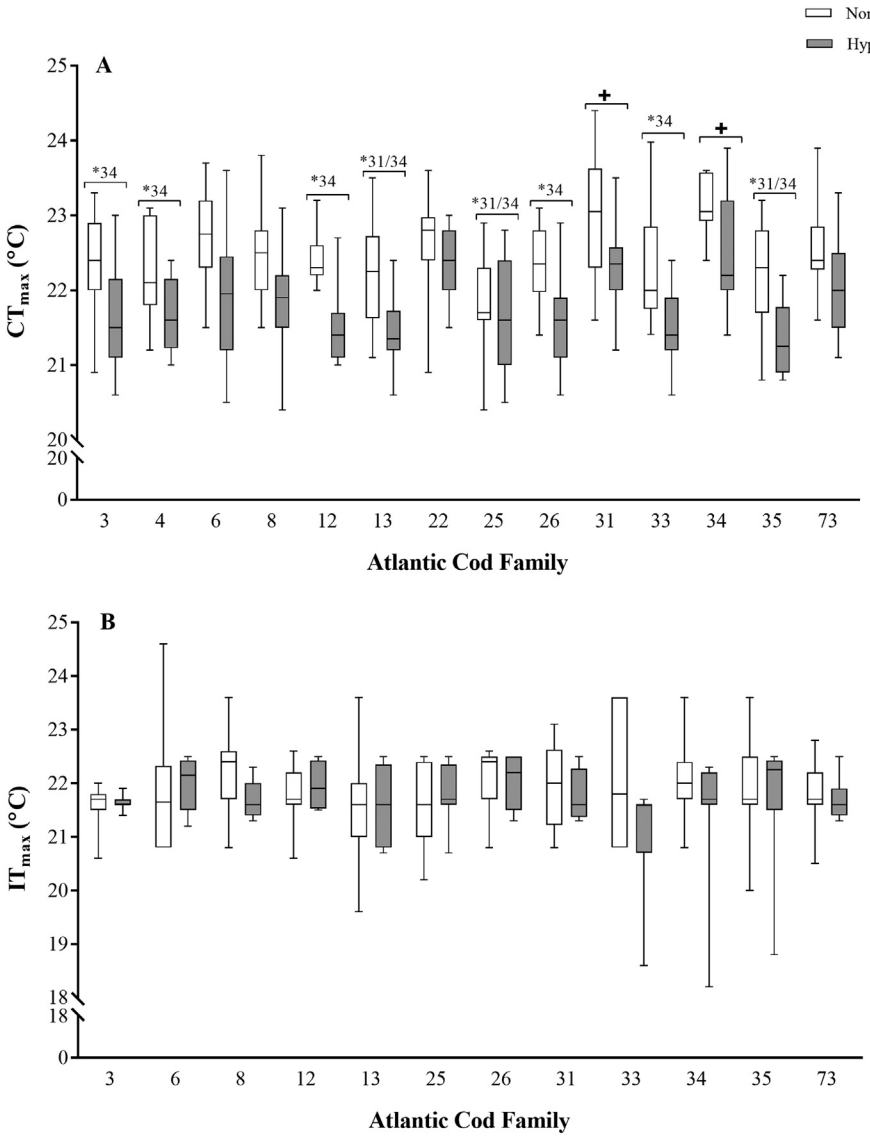

Fig. 1. Upper temperature tolerance values for Atlantic cod (*Gadus morhua*) families challenged with an acute temperature increase (A, 2 °C h^{-1} from 10 °C, N = 27–34) or an incremental temperature increase (B, 1 °C every 4 days from 10 °C, N = 18–28) under normoxia or mild hypoxia (75% air saturation). Significant differences in thermal tolerance ($P < 0.05$) were only found in the CT_{max} experiment. The two families with the highest CT_{max} values (31 & 34; see + over CT_{max} values) were compared with the other families and statistical differences are indicated by * followed by 31 and/or 34. For each family, the horizontal line is the median value, the box delimits the 1st and 3rd quartiles, while the whiskers (error bars) show the 5th and 95th percentiles.

of similar size and acclimated to the same initial temperature (10 °C) in both studies, and the lower IT_{max} reported in Perez-Casanova et al. (2008b) may be partially related to their slower rate of temperature increase (1 °C every 5 days). Nonetheless, both these studies show that increasing temperature over days vs. hours results in a reduced upper thermal tolerance. This is not surprising as many studies (e.g., Becker and Genoway, 1979; Elliott and Elliott, 1995; Mora and Maya, 2006; Moyano et al., 2017) show that the upper thermal tolerance of fishes is higher when water temperatures are increased rapidly. Further, while the rate of temperature increase during the IT_{max} experiment (1 °C every 4 days) should have allowed for compensatory thermal acclimation responses (which take 1–4 weeks for a 10 °C change depending on the physiological function; e.g., see Sandblom et al., 2014; Fu et al., 2018), any gain in thermal tolerance that occurred during the incremental temperature increase may have been offset by the increased thermal stress associated with the long exposure time at each test temperature (Cocking, 1959; Beitinger et al., 2000). In other words, prolonged exposure to thermal stress, beyond pejus limits, may undermine the fish's physiological status and result in the exhaustion of adaptive mechanisms and a reduced upper thermal tolerance. Finally, although the cod greatly reduced their feed intake at temperatures > 17 °C, this is unlikely to have impacted their thermal tolerance. Perez-Casanova et al. (2008a) showed that temperatures at/or near the IT_{max} of this species do not result in a decrease in circulating glucose levels.

The level of hypoxia used in this study (~75% air saturation) was chosen to reflect what may be experienced under sea-cage aquaculture conditions in the North Atlantic (Johansson et al., 2007; Burt et al., 2012). Here, we showed that mild hypoxia decreased the cod's CT_{max} by 0.7 °C. Based on the OCLTT concept (Pörtner, 2002, 2010; Pörtner et al., 2017; but see Jutfeldt et al., 2018) and data which shows that hypoxia constrains maximum metabolic rate and aerobic scope in fishes (including cod: Chabot and Claireaux, 2008; Claireaux and Chabot, 2016), and considering that the metabolic rate of juvenile Atlantic cod increases by approximately 2.6-fold from 10 to 22 °C (Gamperl et al., 2009), a reduction in CT_{max} was not unexpected. Anttila et al. (2015) also demonstrated that moderate overnight (63% air saturation) hypoxia decreased the CT_{max} of Arctic char (*Salvelinus alpinus*) and landlocked salmon (*Salmo salar*) slightly; by 0.2 and 0.4 °C, respectively. Although mild hypoxia decreased the cod's CT_{max}, it had no impact on their IT_{max}. This study is in agreement with recent research conducted by our group on the incremental thermal tolerance of Atlantic salmon (Zanuzzo et al., unpubl.), and we mainly attribute this finding to hypoxic-induced changes over the incremental temperature protocol. For example, Petersen and Gamperl (2011) demonstrated that hypoxia-acclimated (~40% air saturation) Atlantic cod had higher blood hematocrit and hemoglobin levels, and increased tissue oxygen extraction,

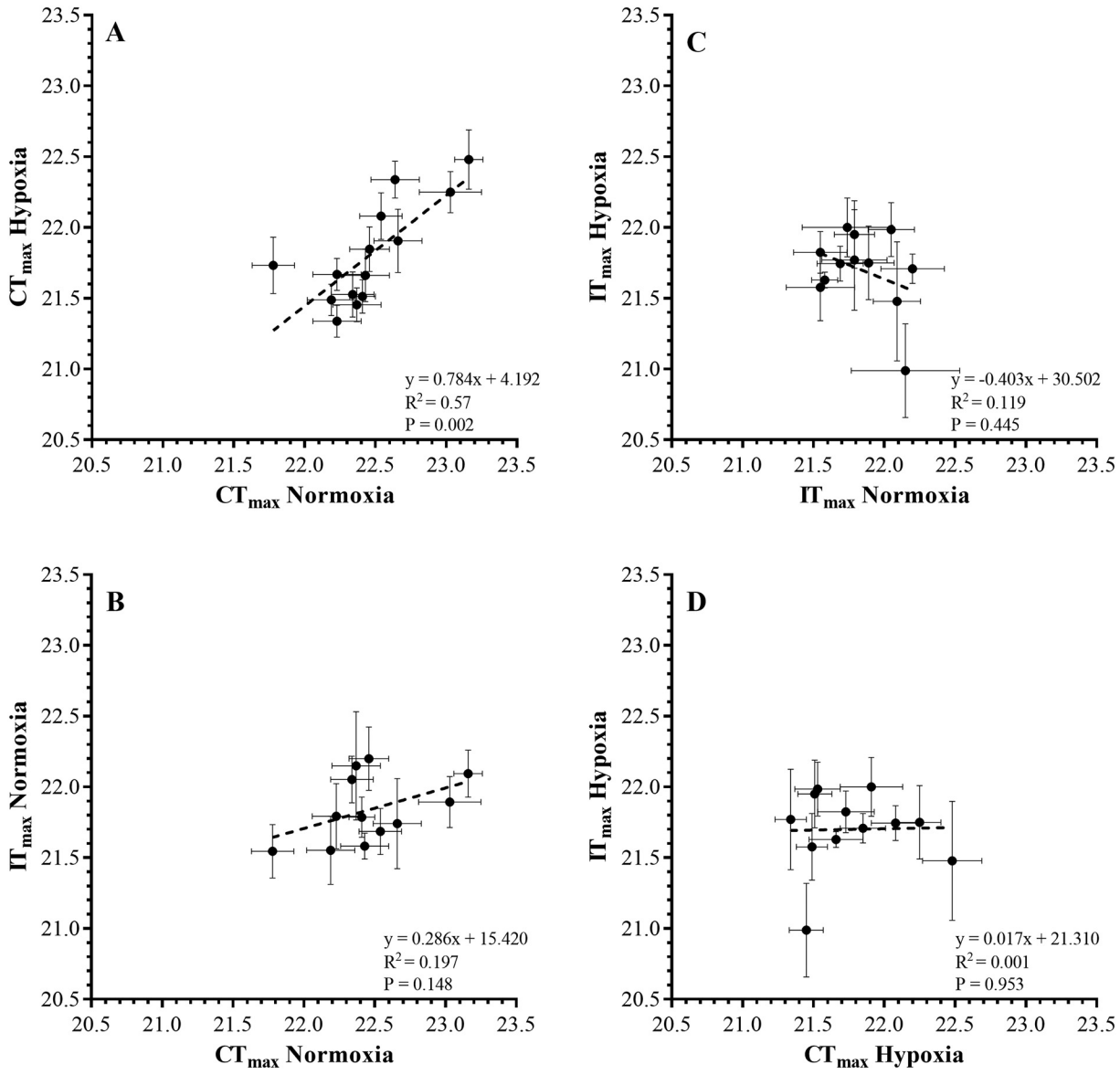

Fig. 2. Correlations between measures of upper thermal tolerance (CT_{max} and IT_{max}) in the Atlantic cod (*Gadus morhua*) families when tested under normoxia and mild hypoxia (75% air saturation). The relationship between CT_{max} under normoxia and mild hypoxia was significant. However, the other three correlations were not significant. Each point represents the mean ± S.E. for a different cod family, N = 12–14.

and the former results are consistent with our recent data on Atlantic salmon challenged with an IT_{max} test to 23 °C. Salmon challenged with an IT_{max} test at ~70% air saturation had higher blood hematocrit levels and hemoglobin concentration than control (12 °C, normoxic) fish, whereas these differences were not significant for fish challenged with the IT_{max} test under normoxia (Zanuzzo et al., unpubl.). Further, functional genomic (i.e., microarray) analysis revealed that 994 different genes were dysregulated (up- or down-regulated) in hypoxia-acclimated salmon as compared to normoxia-acclimated fish after they reached 20 °C during the IT_{max} test (Beemelmanns et al., unpubl.). Thus, we suggest that long-term exposure to mild hypoxia, in combination with increasing temperature, results in a suite of physiological and other responses that enable them to withstand similar temperatures as normoxia-exposed fish.

4.2. Family-based differences in thermal tolerance

Anttila et al. (2013) exposed 41 Atlantic salmon (*Salmo salar*) families to acute temperature increases (CT_{max} challenge at 6 °C h^{-1}) and a hypoxic challenge (1.5% air sat. min^{-1}), separately. These authors showed that both upper temperature (CT_{max}) and hypoxia tolerance varied significantly amongst the families, and that there was a significant correlation between CT_{max} and hypoxia tolerance. The current study also demonstrated a difference in CT_{max} between cod families, and that the CT_{max} of the families was positively correlated when determined under normoxic and hypoxic conditions. Although little is known about the genetic basis of thermal and hypoxia tolerance, some studies have suggested that these traits may be partially determined by genetics, and therefore are heritable (Meffe et al., 1995; Perry et al., 2001; Perry et al., 2005; Doyle et al., 2011; Eliason et al., 2011; Anttila et al., 2013). Perry et al. (2001) and Somorjai et al. (2003) demonstrated that there are quantitative trait loci associated with maximum thermal tolerance (CT_{max}) in rainbow trout (*Oncorhynchus mykiss*) and Arctic char, respectively. CT_{max} has been shown to be heritable in killifish (*Heterandria formosa*) (Doyle et al., 2011) and mosquitofish (*Gambusia holbrooki*) (Meffe et al., 1995). In addition, hypoxia tolerance may be partly determined by genetics in fish, as demonstrated by Nagy et al. (1980) in common carp (*Cyprinus carpio*) and by Collins et al.

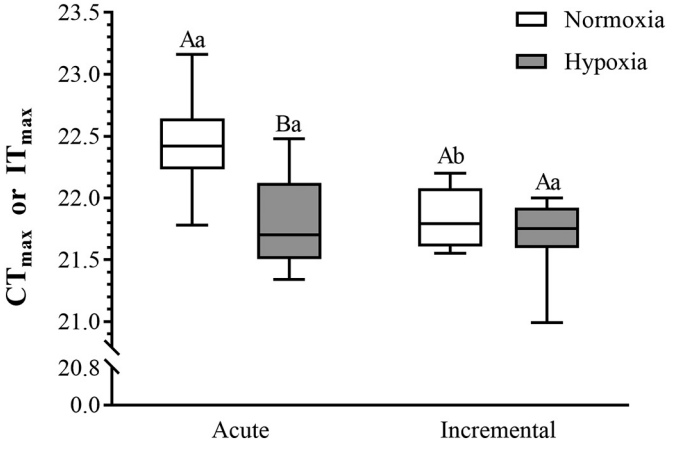

Fig. 3. CT_{max} and IT_{max} values for the Atlantic cod (*Gadus morhua*) families when tested under normoxia and mild hypoxia (75% air saturation). Different capital letters indicate a difference ($P < 0.05$) between normoxia and mild hypoxia within the same thermal challenge, whereas dissimilar lower case letters indicate a difference between the two thermal challenges under normoxia or mild hypoxia. For each family, the horizontal line is the median value, the box delimits the 1st and 3rd quartiles, while the whiskers (error bars) show the 5th and 95th percentiles. N = 12–14.

(2013) for barramundi (*Lates calcarifer*).

This is the first study to investigate the thermal tolerance of fish families exposed to both acute and incremental temperature increases. Given similarities in the nature of changes/responses in cardiac function and oxygen delivery capacity to acute temperature increases and temperature acclimation (Eliason and Anttila, 2017), and that heart size and cardiac function are key determinants of thermal tolerance (Anttila et al., 2013; Farrell, 2009; Farrell et al., 2009), we expected that there would also be a family-based difference in IT_{max} and/or a positive correlation between the upper thermal tolerance as determined by the CT_{max} and IT_{max}. Interestingly, neither of these assumptions were supported by the data (see Figs. 1 and 2), and the explanation for this is not entirely clear. However, Vergauwen et al. (2010) using a microarray approach showed that the responses to an acute thermal challenge are related to the cellular and organismal stress response, an upregulation of defense mechanisms, tissue regeneration and a restructuring of oxygen supply, whereas an incremental temperature increase dysregulates genes related to fuel utilization (compensating for the altered metabolic rate) and cell structure and replacement. In addition, Moyano et al. (2017) investigated the CT_{max} of Atlantic herring (*Clupea harengus*) larvae and suggested that different traits were responsible for the different patterns of individual variation during slower and faster warming rates. Thus, we believe that the lack of correlation between CT_{max} and IT_{max} is at least partially due to these differences in the nature of responses to acute vs. incremental temperature increases. However, it does not explain why there was no significant effect of 'family' on IT_{max}. It is possible that the lower number of fish and families (i.e., 14 vs. 12) used in the IT_{max} experiment, and that IT_{max} showed a non-normal distribution and higher variation (see Fig. 2S) as compared to CT_{max}, could have contributed to the absence of family-based differences in the incremental thermal challenge. This would be in agreement with what other researchers have observed in fish (Moyano et al., 2017) and insects (Chown et al., 2009). Nonetheless, our findings raise an intriguing question; e.g., to what extent is a fish's ability to tolerate incremental temperature increases (as seen at aquaculture cage-sites) related to genetic makeup vs. plasticity in how it is able to modulate gene expression and physiologically important processes as temperatures warm?

4.3. Relationships between temperature tolerance, food consumption and SGR

In this study, we showed that cod decreased feed intake by approximately 50% from 17 to 18 °C, and ceased feeding completely by 21 °C during the IT_{max} test (Fig. 5). The temperature at which our fish began to reduce their feed intake corresponds very well with data on when this species first experiences stress and/or physiological limitation(s) when exposed to elevated temperatures. For example, Lannig et al. (2004) showed that Atlantic cod from the North Sea begin to show cardiac arrhythmias at 16.6 °C during an acute temperature challenge (1 °C h^{-1}). Further, plasma cortisol levels are elevated by 16 °C in 10 °C acclimated Atlantic cod given both CT_{max} and IT_{max} thermal challenges (Perez-Casanova et al., 2008a; Gamperl et al., 2009). In our IT_{max} experiment mortalities began at 21 °C and nearly all fish had succumbed by 22 °C (see Fig. 3S B). Thus, it is no surprise that they had stopped feeding by this temperature.

Although a negative linear regression between feed intake and water O_2 levels has been reported for cod (Chabot and Dutil, 1999; Thorarensen et al., 2017), mild hypoxia had no impact on feed intake in this study. This may seem surprising as, according to Fry (1971), hypoxia may reduce the fish's metabolic scope, and thus, leave less energy available for demanding processes such as specific dynamic action (SDA) and growth. Further, Thorarensen et al. (2017) exposed 10 °C acclimated cod to various levels of hyperoxia and hypoxia for 98 days, and showed that fish subjected to 81 and 65% of air saturation ate 9% and 24% less than the normoxia group. There may be several reasons for the discrepancy. First, the fish in our experiment were initially fed at 1.5% of BW day^{-1}, not to satiation twice daily as in Thorarensen et al. (2017). Fish fed at levels less than satiation may not show decreases in food consumption at mild levels of hypoxia. Nevertheless, Imsland et al. (2005) demonstrated that satiation of juvenile of Atlantic cod held at 13 or 16 °C was achieved at approx. 1.5% BW day^{-1}. Second, our study involved changes in both water oxygen level and temperature, and it is possible that increases in the latter offset any effects of mild hypoxia at the ration the cod were provided.

There is little available information on the mechanisms regulating environmentally-induced changes in feeding and appetite in fishes (Volkoff et al., 2010). However, a number of appetite regulating factors have been identified in cod (Volkoff, 2016), and our results (high temperatures reduce feeding, but no effect of hypoxia; Fig. 5) are consistent with studies that have examined the effect of these two environmental conditions on the neuroendocrine control of feeding in fishes. Hevroy et al. (2012) reported suppressed plasma ghrelin levels, and brain mRNA levels of NPY (neuropeptide Y) and CART (cocaine- and amphetamine-regulated transcript), in adult salmon held at 19 °C vs. 14 °C and suggested that ghrelin was the main signaling mechanism suppressing appetite at the higher temperature; although subsequent research has suggested that increases in the anorexigenic hormone leptin also play a role in decreasing food consumption at high temperatures (Kullegren et al., 2013). Whereas, hypoxia (~72% air saturation) at 17 °C had no effect on salmon plasma ghrelin levels (Vikesa et al., 2017), and neither the mRNA expression of CART or NPY in the hypothalmus of this species were affected by intermittent hypoxia at 10 °C (Burt et al., 2013).

The cod were held for an average of 117 days (~4 months) prior to the CT_{max} experiments and the specific growth rate of the families over this period averaged 0.89 ± 0.03% day^{-1} (mean ± S.E.), and ranged from ~ 0.7 to 1.17% day^{-1} (Fig. 4). The mean value of SGR at 10 °C for the families used in this experiment fits fairly well with the comprehensive growth data of Bjornsson et al. (2001) for Atlantic cod. At 10 °C, they determined that the SGR of 29 g cod fed to satiation was ~2% BW day^{-1}, whereas that of 109 g fish was ~0.8% BW day^{-1}. In our experiment the cod were fed at a ration of 1.5% BW day^{-1} and their average mass over the period that SGR was calculated was ~50 g (see Table S1). Importantly, there was no correlation between the CT_{max} of

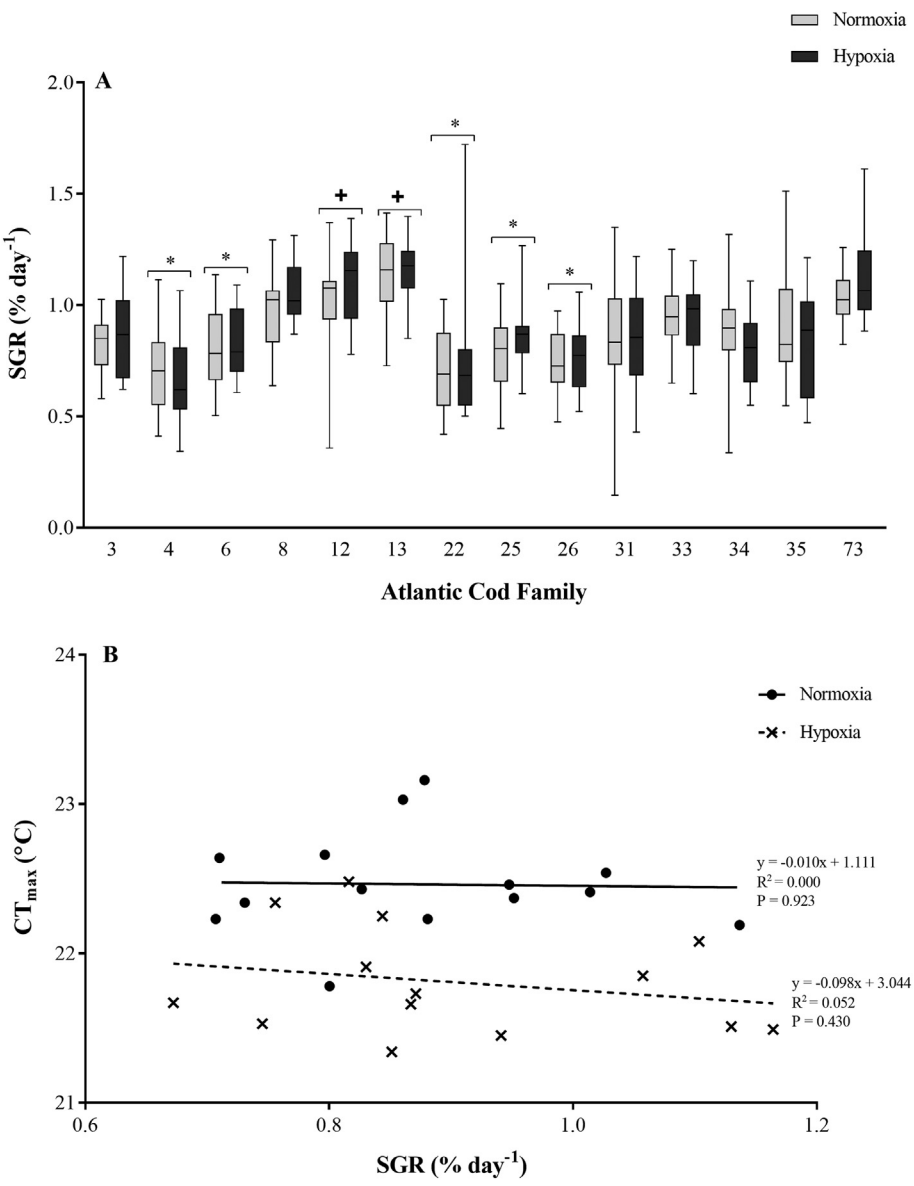

Fig. 4. (A) Specific growth rate (SGR, % day $^{-1}$) of Atlantic cod (*Gadus morhua*) families before they were challenged with an acute temperature increase (CT_{max} test; 2 °C h^{-1} from 10 °C) under normoxia and mild hypoxia (75% air saturation). The two families with the highest SGR (12 & 13; see + over SGR values) were compared with all the other families and statistical differences ($P < 0.05$) are indicated by *. For each family, the horizontal line is the median value, the box delimits the 1st and 3rd quartiles, while the whiskers (error bars) show the 5th and 95th percentiles. (B) Relationship between the mean CT_{max} and SGR of the Atlantic cod families that were challenged with an acute temperature increase under normoxia and mild hypoxia (75% air saturation). No significant ($P < 0.05$) correlation was observed under normoxia or mild hypoxia.

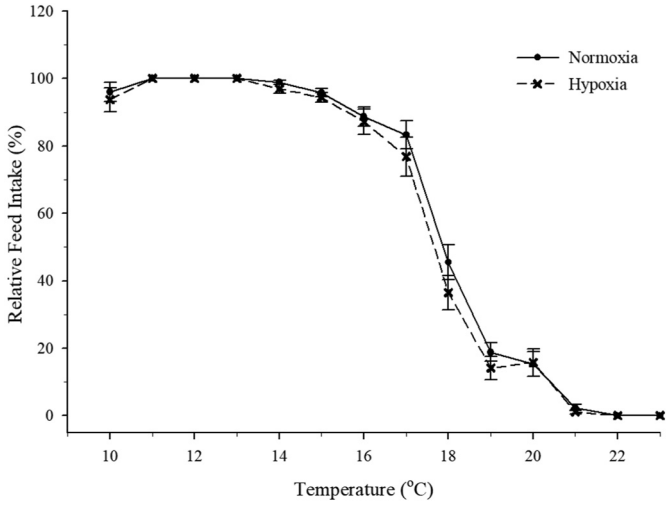

Fig. 5. The effect of an incremental temperature increase (1 °C every 4 days) on relative feed intake (as mean percent of initial ± S.E.) for Atlantic cod (*Gadus morhua*) held under normoxia and mild hypoxia (75% air saturation).

the families and their SGR under conditions of normoxia ($P = 0.92$) or mild hypoxia ($P = 0.43$) (Fig. 4). These data suggest that selecting fish (broodstock) based on CT_{max} will not necessarily enhance the growth rate of a population/stock of Atlantic cod. Given that growth rate is a very important trait that factors prominently into the calculation of breeding values, and that fish will rarely experience rapid (acute) temperature changes that approach their CT_{max} at sea-cage sites, these data question the relevance of incorporating this parameter into breeding programs.

5. Conclusions

In this study, we measured the acute (CT_{max}) and incremental (IT_{max}) thermal tolerance of juvenile Atlantic cod from a number of families under conditions of normoxia and mild hypoxia. This study revealed that the CT_{max} and IT_{max} of this species differ by < 1 °C and are in excess of 20 °C, but that these parameters were not correlated and that CT_{max} did not correlate with SGR. Collectively, these data suggest that while there may be a genetic influence on CT_{max}, it might be difficult to mitigate the impact of climate change-related increases in sea-cage temperatures on the survival and performance of Atlantic cod through selective breeding for this parameter. Interestingly, although

mild hypoxia decreased the cod's CT_{max}, it had no impact on the thermal tolerance (IT_{max}), cumulative mortality or feed intake of Atlantic cod challenged with an incremental temperature increase. This suggests that there is some phenotypic plasticity that this species can recruit to deal with the combined effects of elevated temperatures and mild hypoxia. Further studies should be designed to specifically investigate what trait(s) affect the acute and incremental upper thermal tolerance of Atlantic cod, and the mechanisms that allow cod to compensate when simultaneously exposed to high temperatures and reduced oxygen levels.

Declarations of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cbpa.2019.03.020.

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